

**VERSION OF AMENDED CLAIMS SHOWING THE CHANGES MADE:**

2. (Amended) The method of claim [1] 41, wherein the replication defective hepadnavirus particles are human hepatitis B virus particles
3. (Amended) The method of claim [1] 41, wherein the heterologous gene replaces [is inserted into the region of the S-promoter such that the nucleotides encoding at least one amino acid of an S protein are fused in frame to the 5' end of the heterologous gene] sequences of the S-gene.
4. (Amended) The method of claim [1] 41, wherein the heterologous gene replaces a region of the S-gene under control of the endogenous S-promoter.
5. (Amended) The method of claim [1] 41, wherein the heterologous gene is inserted such that one of [after] an authentic AUG codon of the S-gene or [and the heterologous gene is inserted such that] nucleotides encoding [at least one amino acid] further amino acids of the S-protein are fused in frame to the 5'end of the heterologous gene.
6. (Amended) The method of claim [1] 41, wherein the heterologous gene encodes a modulating agent.

8. (Amended) The method of claim 7, wherein the cytokine is selected from the group consisting of IFN $\alpha$ , IFN $\beta$ , IFN $\gamma$ , TNF $\alpha$ , IL-12 and IL-18.
33. (Amended) A replication defective hepadnavirus particle, wherein a region of the pre-S [or] and S-gene of the hepadnavirus genome [has] have been deleted and replaced [with] by a [therapeutic] heterologous gene such that [expression of the gene is regulated by regulatory sequences of the pre-S or S-gene] the sequences for RC and RII that are essential for producing reverse transcriptase are retained.
34. (Amended) The replication defective hepadnavirus particle of claim 33, wherein the [therapeutic] heterologous gene is a cytokine
36. (Amended) The replication defective hepadnavirus particle of claim 34, wherein the cytokine is selected from the group consisting of TNF $\alpha$ , IFN $\beta$ , IL-18, [and] IFN- $\gamma$  and IL-12.
37. (Amended) A pharmaceutical composition comprising [the replication defective hepadnavirus of claim 33] :
- a replication defective hepadnavirus with a region of its pre-S-genes deleted and replaced with a heterologous gene such that the sequences of the RC r RII that are essential for producing reverse transcriptase are retained, and
  - a pharmaceutically acceptable carrier.

38. (Amended) The pharmaceutical composition comprising [the replication defective hepadnavirus of claim 33] :

- a replication defective hepadnavirus with a region of its pre-S-gene deleted and replaced with a heterologous gene that the sequences of the RC r RII that are essential for producing reverse transcriptase are retained, and
- a helper virus

39. (Amended) A method of producing [therapeutic] replication defective hepadnavirus particles at a titer suitable for [therapeutic use] infecting hepatocytes comprising:

- co-transfecting hepatocyte cells of a hepatoma cell line [hepatoma cell lines] with:
  - (i) replicating defective hepadnavirus constructs, wherein a region of one of a pre-S or an S-gene of the hepadnavirus DNA has been replaced with a gene encoding a [therapeutic] heterologous gene while retaining one of an RC or RII signal, such that the expression of the gene encoding a cytokine is regulated by regulatory sequences of the [pre-S] S-gene; and
  - (ii) a helper construct for transcomplementing lacking viral gene products;

- culturing the hepatocytes until infectious viral particles are produced; and
- recovering the infectious particles.

## REMARKS

The last Office Action of February 14, 2001 has been carefully considered. Reconsideration of the instant application in view of the foregoing amendments and the following remarks is respectfully requested.

Claims 1-40 are pending in the application.

It is noted that claims 1-8 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

It is further noted that claims 9-40 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 9-32 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-2, 4, 6, 33 and 37 stand rejected under 35 U.S.C. §102(b) as being anticipated by U.S. Pat. No. 5,981,274 (hereinafter "Tyrrell").

Claims 1, 3, 5-8, 33-36. stand rejected under 35 U.S.C. §103(a) as being unpatentable over Tyrrell in view of PNAS reference 91: 1198-1205 (hereinafter

"Guttermann") in view of J. of Virology 71 :3236-32-43, 1997 reference (hereinafter "Cavanaugh").

## **OBJECTION TO THE CLAIMS**

Claim 11 has been corrected to obviate the objection;

Claim 30 has been cancelled;

Claim 35 has been corrected with respect to IFN $\alpha$ .

In view of these corrections, it is believed that the Examiner's objections have been obviated.

## **REJECTION OF CLAIMS 1-8 AND 9-40 UNDER 35 U.S.C. §112, FIRST PARAGRAPH**

Applicants has cancelled claims 9-32, amended claims 33 to 40 and presented new claims 41-50. Claims 2, 3, 4, 5, 6, and 8 were amended to correct the dependency and also to clarify what applicants consider to be their invention.

The Examiner has rejected the claims under 35 U.S.C. Section 112 first paragraph as not being supported by the disclosure. In response to this rejection applicants have cancelled claims 9 through 32 to obviate this rejection. Applicant's further presented new claims 41-50 in view of the Examiners' position. Claim 42 as now presented is directed to a method for producing replication defective recombinant hepadnavirus particles capable of expressing a

heterologous gene in hepatocytes. Support of the subject matter claimed is found in the specification and has been shown in experiments as set forth on page 19 beginning line 37 under the heading HBV Methodologies in conjunction with Example 8. The HBV infection and gene transfer is likewise supported in the specification at page 20, line 11 through 14. An immuno fluorescence staining technique was carried out to test for the intracellular HBV antibody. Example 9 shows that GFP fluorescence was detected. Since claims 9 through 32 have been cancelled the Examiner's comments targeted on treatment of a subject has been eliminated. Furthermore, all references in the claims a therapeutic gene has been likewise eliminated. As now presented, claim 42 and the claims depending therefrom is narrowly directed to infecting hepatocytes, the subject matter of which is fully supported by the specification.

In view of presenting the new claims 41-50 and claims 9-32 having been cancelled, the claims are fully supported by the specification.

Withdrawal of the rejection of claims 1-8 and 41-50 under 35 U.S.C. §112, first paragraph is thus respectfully requested.

#### **REJECTION OF CLAIMS 9-32 AND 39-40 UNDER 35 U.S.C. §112, SECOND PARAGRAPH**

The Examiner's rejection of claims 9-32 has been obviated by virtue of these claims being cancelled.

With respect to claims 39-40, the Examiner's rejection is respectfully traversed in view of the amendment made to these claims. In particular, claim 39

has been amended to delete all references that the method is directed to therapeutic use. Since claim 40 depends on claim 39 these references apply to this claim also. In view of this amendment, applicants believe the rejection has been obviated.

Withdrawal of the rejection of the claims 39-40 under 35 U.S.C. §112 second paragraph is thus respectfully requested.

**REJECTION OF CLAIMS 1-2, 4, 6, 33, AND 37 UNDER 35 U.S.C. §102 AS  
BEING ANTICIPATED BY TYRRELL**

The Examiner's rejection under 35 U.S.C. 102(a) is respectfully traversed.

The Examiner has cited the Tyrrell reference as disclosing the applicants claimed invention.

Tyrrell teaches the preparation of a recombinant HBV genome in which a foreign DNA was integrated into the polymerase open reading frame but, as it appears, under the control of the preS1 promoter. Thus, Tyrrell describes insertion into the HBV genome of a heterologous gene, however, as the Tyrrell experiments show, a possible method that is postulated in the description is supported only by experiments that produce a very different result than the one claimed here. The Tyrrell experiments indicate that there is no replacement, that is, deletion and replacement of the relevant sequences in the HBV genome. The replacement is critical since the length of the genome is critical not only for reverse transcription but also for optimal efficiency in packaging to produce

recombinant viral particles. In contrast, applicants are able to replace the relevant sequences after deletion of the S-gene. While Tyrrell claims deletion of the S/S2 gene, the example does not show this. The examples refer to the pre-S1 gene but not deletion of the S/S2 gene.

Use of the hepadnaviral S-promoter as claimed here drives a message distinct from that driven by the pre-S promoter as used by Tyrrell. Significantly, the cis-acting sequences are essential for efficient reverse transcription, RC or R II respectively. Tyrrell does not teach that the cis-acting sequences have to be preserved, as they are essential for circularization of the pregenomic HBV RNA and for reverse transcription.

Methods disclosed by Tyrrell do delete most of the pol ORF of the HBV genome but do not preserve the any essential cis-acting sequences, therefore these methods are not suitable to generate recombinant viral particles at titers high enough for infection.

It is believed that the claims as amended in view of the foregoing discussion, patentably differentiate over the Tyrrell reference

Withdrawal of the rejection of claims 1-2, 4, 6, 33, AND 37 under 35 U.S.C. §102 is thus respectfully requested.

**REJECTION OF CLAIMS 1, 3, 5-8, 33-36 AND 38 UNDER 35 U.S.C. §103 AS  
BEING UNPATENTABLE OVER TYRRELL IN VIEW OF GUTTERMAN AND  
CAVANAUGH.**

The Examiner has now rejected the original claims as unpatentable over Tyrrell in view of Gutterman and Cavanaugh.

The Examiner's rejection of claims 1, 3 and 5-8, 33-36 and 38 is respectfully traversed.

The Examiner has postulated that " Tyrrell teaches the preparation of replication defective recombinant HBV viruses comprising heterologous gene sequences operably linked to the pre-S<sub>1</sub> promoter for the expression of functional heterologous gene products in liver cells. The Examiner also points to claims 1-3 and 9-12 in Tyrrell.

It should be noted that Tyrrell states their method intends to correct errors in cellular metabolism or kill cancerous cells or deactivate pathogens which is different from the what the current invention is designed to do, namely to administer mediators to stimulate the immune system.

Furthermore, Tyrrell actually does not show expression of the gene. Indeed, Tyrrell do not show that they produce *any* enveloped particles at all. The method disclosed does not differentiate between unenveloped "naked" capsid particles from correctly enveloped virions capable to infect human hepatocytes.

Furthermore due to the method used in Tyrrell are shown far below that observed from parallel transfection of an HBV wildtype genome (see Example 4) and would not be sufficient to for gene transfer vector at all.

Referring to Example 6 under subsection (e) in Tyrrell, it is seen that after infection, all that is shown is the presence of intracellular recombinant HBV vector DNA or RNA, which Tyrrell claims to be the indication that gene transfer took place. However, no other test were done showing that such gene transfer took place and the result is that in that particular experiment DNA and RNA are associated with HepG2 cell of a cell line.

In contrast, in the current application, actual human hepatocytes were infected and the gene expression was tested by the presence of GFP. After 6 days 1 in  $10^2$  hepatocytes were found to be infected. After 12 days 1 in  $10^4$  hepatocytes clearly showed detectable GFP fluorescence.

The Examiner has furthermore discussed Tyrrell as providing an efficient packaging limit for the HBV particle. However, the Tyrrell method does delete most of the pol open reading frame of the HBV genome but does not preserve any essential cis-transacting sequences and therefore not suitable to generate recombinant viral particles at high titers.

The Examiner has admitted that Tyrrell does not teach the preparation of replication defective recombinant HBV viruses comprising a therapeutic gene encoding for cytokine. However, it should be noted that Tyrrell does not show any expression of a gene let alone a gene that produces a therapeutic substance. The Examiner has cited Cavanaugh as showing that it would have

been obvious to an ordinary skilled artisan to insert heterologous genes encoding for IFN $\alpha$ , IFN $\gamma$  and TNF $\alpha$ . when combined with Tyrrell. As already stated above Tyrrell's objective is concerned with correcting cellular metabolic errors, inactivation of pathogens or killing cancerous cells. Thus to use the Examiner's hypothetical construct that Tyrrell discloses such a system which can be combined with the disclosure of Gutterman or Cavanaugh would not lead to the method for vector transfer as claimed here.

Thus the ordinary skilled artisan would not look to Tyrell when informed about Guttermann and Cavanaugh to produce a vector transfer of a gene that *mediates* an immune response but does not fight cancerous cells or inactivate pathogens as such. Gutterman systemic application of cytokines

The advances the claimed invention made were also subject of a published comment by Don Ganem published in PNAS 96 (1999): 11696-11697) following the Protzer *et al* paper (PNAS 96 (1999): 10818-10823)

"Protzer *et al* report a major advance in hepadnaviral vectoring", "the hepadnaviral genome is virtually blanketed with critical cis-acting elements... by diligent screening they have identified a region of the viral genome that evidently lacks important cis-acting sequences and therefore tolerates substitution with foreign DNA"... "work in several laboratories for more than a decade... has vigorously pursued this possibility, but successes have been few."

The Examiner has cited page 3242 lines 8-20 of Cavanaugh, for the proposition that the alpha/beta/gamma interferons are known to inhibit gene

expression. However, when read correctly the passage concludes by stating that it was not understood why HBV replication is not inhibited as expected in fact that IL-12 did not induce cytokines in sufficient quantities or in the proper ratio to inhibit viral gene expression in the treated animals. This clearly undermines the proposition by the Examiner that the Cavanaugh reference when combined with the teachings of Tyrrell renders the claimed invention obvious. In any event the Cavanaugh reference is concerned with systemic application which is an entirely different approach than is carried out here and the two methods cannot be combined.

Cavanaugh *et al* teach that an antiviral immune response in HBV transgenic mice can be initiated by recombinant murine IL-12 applied systemically to the mice. IL-12 is known to be a cytokine with strong immunostimulatory effects. Cavanaugh *et al*. demonstrated that various cytokines were induced in various organs of these mice after IL-12 administration. It is suggested that systemic application of IL-12 may have therapeutic value as a treatment of chronic HBV infection. Cavanaugh's' broad immune activation is directly opposite from the organ specific delivery as claimed here where a systemic immune stimulation is to be avoided.

Gutterman is likewise directed to a systemic application and teaches that a systemic application is the treatment of choice for chronic hepatitis B and C. However, such an application does not combine with the method as disclosed in Tyrrell.

In view of the Examiner's grounds for rejection, applicants submit herewith new claims to clearly point out the features of the present invention. In drafting the new claims, great care has been taken to distinguish the present invention from the invention disclosed by the various references and also to overcome the rejection under 35 U.S.C. §112.

For the reasons set forth above, it is applicant's contention that neither Tyrrell nor Cavanaugh and Gutterman, nor a combination thereof teaches or suggests the features of the present invention, as recited in claims 42, 43, 37, 38, 39.

Claims 2 to 8 which depends from claim 42 and therefore contains all the limitations thereof, patentably distinguishes over the applied prior art in the same manner as claim 42.

It should, however, be noted that these dependent claims contain individual patentable features per se. In this connection, applicant wishes to refer e.g. to claim 41 relating to a helper construct. Thus, applicants not only recognized the main drawback in the prior art but attains also a solution in packaging.

Withdrawal of the rejection of claims 1, 3, 5-8, 33-36 and 38 under 35 U.S.C. §103 and allowance thereof are thus respectfully requested.

## **CITED REFERENCES**

Applicant has also carefully scrutinized the further cited prior art and finds it without any relevance to the newly submitted claims. It is thus felt that no specific discussion thereof is necessary.

## CONCLUSION

Applicant believes that when the Examiner reconsiders the claims in the light of the above comments, he will agree that the invention is in no way properly met or anticipated or even suggested by any of the references however they are considered.

None of the references discloses a method for producing a replication defective recombinant hepadnavirus which expresses the heterologous gene in hepatocytes as claimed. .

In view of the above presented remarks and amendments, it is respectfully submitted that all claims on file should be considered patentably differentiated over the art and should be allowed.

Applicant further submits a new Power of Attorney signed by the inventors.

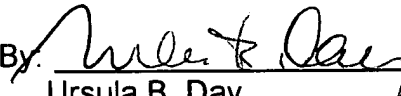
Reconsideration and allowance of the present application are respectfully requested.

Should the Examiner consider necessary or desirable any formal changes anywhere in the specification, claims and/or drawing, then it is respectfully requested that such changes be made by Examiner's Amendment, if the

Examiner feels this would facilitate passage of the case to issuance. If the Examiner feels that it might be helpful in advancing this case by calling the undersigned, applicant would greatly appreciate such a telephone interview.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1747.

Respectfully submitted,

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